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Tuning mTORC1 Activity for Balanced Self-Renewal and Differentiation

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Understanding the mechanisms that control stem cell self-renewal and differentiation are at the core of stem cell biology. In a recent issue of *Cell*, Hobbs et al. report that harnessing mTORC1 activity by the transcription factor PLZF is crucial to maintain the self-renewing activity of germline progenitors.

About 1000 spermatozoa are produced with every breath of an adult man. Similar to the organization of other regenerating tissues such as blood, skin, and intestine, mature spermatozoa are continuously produced by differentiating proliferating progenitors (spermatogonia) that are derived from rare spermatogonial stem cells (SSCs). These stem cells arise after birth, persist throughout the male lifetime, and are estimated to comprise about 1 in 3000 cells per testis. SSCs reside at the base of the seminiferous tubules and are surrounded by Sertoli cells that together with other cells, including Leydig cells, generate a stem cell niche (Oatley and Brinster, 2008).

One of the key niche factors secreted by Sertoli cells is the TGF-β superfamily member glial cell-line derived neurotrophic factor (GDNF), which activates Src family kinases and the PI3-kinase-Akt-mTORC1 pathway in spermatogonial progenitor cell (SPC) (Figure 1). Expression of GDNF is required for normal spermatogenesis, and its overexpression leads to the accumulation of spermatogonia (Meng et al., 2000). Moreover, GDNF activity is essential to maintain spermatogonial progenitor cells (SPC) self-renewal in long-term in vitro cultures (Kubota et al., 2004). Other signaling molecules that appear to further promote SPC self-renewal include bFGF, EGF, and CSF-1. In addition, a number of transcription factors including PLZF, Taf4b, BCL6b, ETV5, and Lhx1 have been suggested to be important for the function of spermatogonial stem/progenitors (Oatley and Brinster, 2008). However, it remains largely unknown how the different signaling components are connected to control the delicate balance between SPC self-renewal and differentiation. In the current issue of *Cell*, Hobbs et al. (2010) now provide insights into this fascinating process by suggesting that the harnessing of mTORC1 activity by PLZF is crucial to promote SPC self-renewal while simultaneously allowing for balanced differentiation of the spermatogonial compartment.

The promyelocytic leukemia zinc finger (PLZF) transcription factor has recently been shown to be a crucial regulator of germ cell function, as PLZF-deficient mice undergo progressive germ cell loss and testis atrophy associated with infertility (Costoya et al., 2004). Taking advantage of PLZF expression in early germline progenitors, Hobbs et al. (2010) identified by FACS a population of cells significantly enriched for SPCs from juvenile testis. Prepubertal PLZF-deficient testis contained only about half as many of these αv-integrin^{neg} Thy-1^{low} c-Kit^{neg} cells as compared to wild-type. Moreover, consistent with the observed progressive loss of fertility in PLZF deficient mice, PLZF-deficient cells show an increase in the c-Kit^{pos} population, indicating the presence of a more proliferative and differentiated population of SPCs.

To dissect the molecular mechanism governing PLZF-mediated self-renewal of SPCs, the authors took advantage of a recently developed long-term SPC culture protocol to generate control and *Plzf*$^{-/-}$ SPCs cell lines. Interestingly, *Plzf*$^{-/-}$ SPCs are physically larger than controls due to hyperactive mTORC1 (mammalian target of rapamycin complex 1). This size phenotype is rescued by treatment of cells with the mTORC1 inhibitor Rapamycin. The authors ruled out an enhanced growth factor response (GDNF, bFGF, and EGF) as a cause of the augmented mTORC1 activity, as both Akt and Erk activities are decreased. In addition, other upstream regulators of mTORC1 such as Tsc1/2, Rheb, or mTOR itself are unchanged in mutant cells. However, a suppressor of mTOR, Redd1, is substantially underexpressed in PLZF deficient SPCs. Redd1 inhibits mTORC1 through regulation of TSC1/TSC2 and is induced by stress stimuli such as hypoxia, DNA damage, and oxidative stress (Ellisen, 2005). The authors further showed that PLZF directly activates the Redd1 promoter, resulting in its increased expression, which mediates subsequent mTORC1 inhibition (Figure 1).

These data explain why mTORC1 activity is increased in PLZF mutants, but how is this associated with progressive loss of SPC activity? Since GDNF signaling is essential for SPC self-renewal, the authors examined whether mTORC1 may negatively influence GDNF-mediated signaling, particularly as PLZF mutants show reduced Akt activity. Indeed, PLZF mutant SPCs show reduced expression of the two GDNF receptor components GFRα1 and c-Ret, which could be reversed by treatment with Rapamycin. Since reduced GDNF signaling leads to loss of SPC self-renewal, these data suggest the presence of a negative feedback loop between mTORC1 and GDNF signaling that controls the balance between self-renewal and differentiation (Figure 1). In normal SPCs, PLZF activates Redd1, which keeps mTORC1 activity low and thus allows GDNF receptor mediated Akt activation and self-renewal. In the absence of PLZF, Redd1 is absent, and mTORC1 becomes hyperactivated,

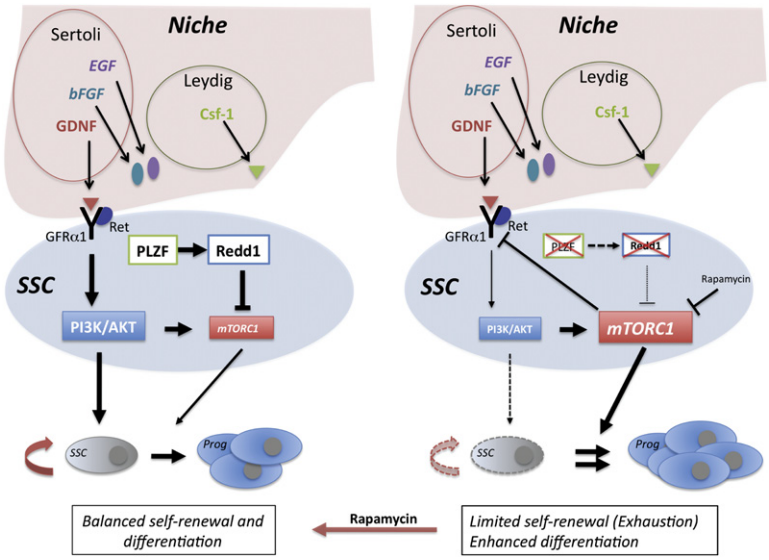

Figure 1. Model of PLZF and mTORC1 Function in Spermatogonial Stem Cell (SSC) Self-Renewal and Differentiation

Left, GDNF secreted by Sertoli cells activates via its receptors (GFRα1 and c-Ret) the PI3K/AKT pathway in SSCs, which signals to mTORC1 to balance self-renewal and proliferation of SSCs. PLZF integrates signaling pathways governing progenitor self-renewal via transcriptional control of Redd1, an mTORC1 inhibitor protein. Right, in the absence of PLZF, mTORC1 activity is enhanced due to decreased expression of Redd1. In this situation, a negative feedback loop from hyperactivated mTORC1 to the GDNF receptor is established. This leads to defective self-renewal and exhaustion of the SSC pool as a result of low PI3K/AKT signaling in response to niche-derived GDNF. As a result, differentiated spermatogonial progenitors (Prog) are transiently increased. The mTORC1 inhibitor Rapamycin is able to rescue the SSC exhaustion phenotype of *Plzf*$^{-/-}$ mice.

followed by downregulation of the GDNF receptor components. This prevents sufficient Akt activation in response to niche-derived GDNF, thus tipping the balance toward differentiation, resulting in the progressive loss of SPCs. In agreement with this model, the authors provided data suggesting that Rapamycin treatment of juvenile mice attenuates the defect in *Plzf*$^{-/-}$ mice, as shown by the restoration of the SPC enriched αv-integrin^{neg} Thy-1^{low} c-Kit^{neg} population. Finally, prolonged Rapamycin treatment of wild-type mice remarkably increases not only SPC frequency, but also GFRα1 and c-Ret expression, providing strong support for the hypothesis that the level of mTORC1 activity controls the balance between SPC self-renewal and differentiation in vivo.

Germline progenitors are not the only cell type in which mTORC1, the central mediator of cell growth, takes center stage in stem cell function. It has previously been shown that hyperactivation of this pathway in hematopoietic stem cells (HSCs) leads to loss of quiescence, followed by stem cell exhaustion (Gan and DePinho, 2009). This is in agreement with the finding that during homeostasis, several stem cell types are maintained in a stage of dormancy associated with reduced metabolism (Wilson et al., 2008; Fuchs, 2009). This may serve as a mechanism to prevent the accumulation of mutations in dangerously potent stem cells, which could otherwise facilitate the initiation of tumorigenesis. While stem cells can be protected by staying transiently dormant, progenitors need to rapidly proliferate and upregulate their metabolic pathways. To counteract the associated risk of progenitor transformation, hyperactive mTORC1 not only drives the metabolism but appears to simultaneously shut-down the self-renewal process. The findings of Hobbs et al. (2010) suggest that this may, at least in part, be mediated by desensitizing progenitors to stem cell niche-derived signals, like GDNF in the case of SPCs (Figure 1).

Although the present work unravels some exciting new regulatory self-renewal pathways and links niche-derived signals via cytoplasmic regulators to nuclear transcription factors, several important questions remain to be addressed. First, novel markers are required to better identify the different stem/progenitor populations present in the mammalian testis. Functional reconstitution assays revealed that the αv-integrin^{neg} Thy-1^{low} c-Kit^{neg} population contains only about 1 functional SSC per 80 cells (Hobbs et al., 2010). Thus, it would be highly relevant to show whether the PLZF-mTORC1 network identified here indeed operates at the most primitive SSC level, or is present only in the pool of progenitors. Second, PLZF seems to be a crucial SPC regulator upstream of mTORC1, but how its expression is controlled in SPCs remains unclear. Third, is the identified PLZF-Redd1-mTORC1 pathway also used by other types of stem cells? As PLZF is also highly expressed in human hematopoietic stem/progenitors, where it restricts proliferation and differentiation of myeloid progenitors, this seems a likely possibility (Doulatov et al., 2009).

Although the regulation of stem cell self-renewal involves the interaction of many cell type-specific regulatory circuits, some players like mTORC1 may be commonly used by most stem cells. The identification of more elements controlling TORC1 activity will further enhance our understanding of normal and malignant stem cells.

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